* May contain prev. viewed cites.

(FILE 'HCAPLUS' ENTERED AT 16:06:42 ON 06 DEC 2002)
L1 38 SEA FILE=HCAPLUS ABB=ON PLU=ON SEX##(5A)(SORT? OR SELECT? OR PRESELECT? OR PREDETERM? OR PRE DETERM?) AND SPERM?
L2 1 SEA FILE=HCAPLUS ABB=ON PLU=ON L1 AND OVINE

L2 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:391796 HCAPLUS
TITLE: Method of cryopreserving selected **sperm** cells
INVENTOR(S): Schenk, John
PATENT ASSIGNEE(S): Xy, Inc., USA
SOURCE: PCT Int. Appl.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001037655	A1	20010531	WO 2000-US30155	20001122
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 2000016049	A	20020813	BR 2000-16049	20001122
EP 1257168	A1	20021120	EP 2000-980267	20001122
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 1999-167423P P 19991124
US 2000-478299 A 20000105
WO 2000-US30155 W 20001122

AB The present invention provides a method of cryopreserving **sperm** that have been selected for a specific characteristic. In a preferred embodiment, the method is employed to freeze **sex-selected sperm**. Although the cryopreservation method of the invention can be used to freeze **sperm** selected by any number of selection methods, selection using flow cytometry is preferred. The present invention also provides a frozen **sperm** sample that has been selected for a particular characteristic, such as sex-type. In preferred embodiments, the frozen **sperm** sample includes mammalian **sperm**, such as, for example, human, bovine, equine, porcine, **ovine**, elk, or bison **sperm**. The frozen selected **sperm** sample can be used in a variety of applications. In particular, the sample can be thawed and used for fertilization. Accordingly, the invention also includes a method of using the frozen selected **sperm** sample for artificial insemination or in vitro fertilization.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR

THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, VETU, VETB, CABA, AGRICOLA' ENTERED AT 16:07:50 ON 06 DEC 2002)

L3 6 S L2
L4 6 DUP REM L3 (0 DUPLICATES REMOVED)

L4 ANSWER 1 OF 6 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-442142 [47] WPIDS
DOC. NO. NON-CPI: N2001-327014
DOC. NO. CPI: C2001-133742
TITLE: Producing high-genetic value embryos of specified sex, comprises thawing frozen solution of genetically-selected **spermatozoa**, sorting them into subpopulations, and fertilizing oocytes with subpopulation in vitro.
DERWENT CLASS: B04 C06 D16 P14 P32 S03
INVENTOR(S): BETTIO, D; BETTIO, L; ALEANDRI, R; GALLI, A
PATENT ASSIGNEE(S): (BETT-N) BETTIO GROUP SRL; (SPER-N) IST SPERIMENTALE ITAL LAZZARO SPALLANZAN
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001051612	A1	20010719	(200147)*	EN	31

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

FR 2806441	A3	20010921	(200160)
AU 2001030176	A	20010724	(200166)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001051612	A1	WO 2001-EP281	20010111
FR 2806441	A3	FR 2001-3213	20010309
AU 2001030176	A	AU 2001-30176	20010111

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001030176	A	Based on	WO 200151612

PRIORITY APPLN. INFO: IT 2000-MI30 20000114; IT 2000-TV30 20000314
AN 2001-442142 [47] WPIDS
AB WO 200151612 A UPAB: 20010822
NOVELTY - Producing high-genetic value embryos of **predetermined sex** derived from gametes of

non-human mammalian species, comprising thawing and diluting a frozen solution of genetically-selected **spermatozoa** (GS), staining GS with a DNA specific fluorescent dye, sorting GS into subpopulations (SP), in vitro homologous fertilization of oocytes with SP, and incubating fertilized oocytes in mammalian cell culture medium, is new.

DETAILED DESCRIPTION - Producing high-genetic value embryos of **predetermined sex** derived from gametes of non-human mammalian species, comprising:

(a) thawing a frozen solution of GS and diluting it;

(b) staining the **spermatozoa** with a DNA specific fluorescent dye;

(c) diluting out the stained sample;

(d) sorting the stained **spermatozoa** population by flow-cytometry into a **spermatozoa** subpopulation (SP1) containing chromosome X and a **spermatozoa** subpopulation (SP2) containing chromosome Y, using a protein free solution as sheath fluid;

in vitro homologous fertilization of oocytes with SP1 or SP2; and

(e) incubation of fertilized oocytes into a mammalian cell culture medium, is new.

An INDEPENDENT CLAIM is also included for a non-human high-genetic value mammalian embryo of **predetermined sex**, obtainable by the above said method.

USE - The method is useful for producing high-genetic value embryos of **predetermined sex** derived from gametes of non-human mammalian species selected from **ovine**, bovine, equine and rodents (claimed). The method is useful in the zootechnical industry.

ADVANTAGE - Non-human mammalian embryos produced by the above said method is of **predetermined sex** and has high-genetic value. The method is effective for the production of embryos from frozen semen. The method allows the choice of the individual carrying the useful genetic characteristics without time and geographical limitations, because of the use of the frozen semen. The possibility to sex frozen semen allows more efficient production of progeny aimed at particular purposes in the zootechnical industry, or addressed to particular production lineage. The method represents an important technical achievement by using frozen semen, over the prior art methods where sexing is performed on fresh semen. Potentially any semen with desired characteristics can be used in any country and at any time, i.e., potentially without any time and place limit imposed by the use of fresh semen. The method allows a fertilization efficiency still suitable for industrial purposes. The embryos having high-genetic value, can be produced entirely in the laboratory, where they can be frozen and used in the breeding centers.

Dwg.0/4

L4 ANSWER 2 OF 6 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-387811 [33] WPIDS
CROSS REFERENCE: 1999-394774 [33]; 2000-387812 [33]
DOC. NO. NON-CPI: N2000-290279
DOC. NO. CPI: C2000-117815
TITLE: Transfecting male germ cells for producing transgenic mammals, comprises depopulating vertebrate testis of male germ cells and

transfecting with polynucleotide encoding desired trait.

DERWENT CLASS: B01 B04 B05 C03 C06 D16 P14

INVENTOR(S): READHEAD, C W; WINSTON, R; KOEFFLER, H P; MULLER, C

PATENT ASSIGNEE(S): (CEDA-N) CEDARS SINAI MEDICAL CENT; (UNLO) IMPERIAL COLLEGE SCI TECHNOLOGY & MED; (KOEF-I) KOEFFLER H P; (MULL-I) MULLER C; (READ-I) READHEAD C W; (WINS-I) WINSTON R

COUNTRY COUNT: 87

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000029601	A1	20000525	(200033)*	EN	61
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9940771	A	20000605	(200042)		
EP 1047792	A1	20001102	(200056)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
US 2002056148	A1	20020509	(200235)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2000029601	A1		WO 1999-US10573	19990513
AU 9940771	A		AU 1999-40771	19990513
EP 1047792	A1		EP 1999-924219	19990513
			WO 1999-US10573	19990513
US 2002056148	A1	Provisional	US 1997-65825P	19971114
		CIP of	US 1998-191920	19981113
		Div ex	US 1999-292723	19990415
			US 2001-919042	20010730

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9940771	A	Based on	WO 200029601
EP 1047792	A1	Based on	WO 200029601
US 2002056148	A1	CIP of	US 6316692

PRIORITY APPLN. INFO: WO 1999-US8277 19990415; US 1998-191920 19981113; WO 1998-US24238 19981113; US 1999-292723 19990415

AN 2000-387811 [33] WPIDS

CR 1999-394774 [33]; 2000-387812 [33]

AB WO 200029601 A UPAB: 20020603

NOVELTY - In vitro (I) and in vivo transfection of male germ cells with a polynucleotide (PN) encoding a desired trait or product, is new. (I) comprises transfecting germ cells from a donor male vertebrate with PN and administering the cells the a testis of a recipient male vertebrate which is previously depopulated of genetically unaltered male germ cells by an alkylating agent or

gamma irradiation.

DETAILED DESCRIPTION - The method further comprises causing the administered germ cells to lodge in the seminiferous tubule of the recipient male vertebrate. In vivo transfection (II) involves administering to a male vertebrate's testis a gene delivery mixture comprising PN and at least 1 lentiviral-derived transfecting or gene delivery agent, and optionally a genetic selection marker, under conditions effective to reach the vertebrate's germ cells or precursors and causing the PN to be taken up by, and released into, germ cells or precursors.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of depopulating (III) a vertebrate testis of germ cells, comprising administering a dose of an alkylating agent to a male vertebrate and gamma -irradiating;

(2) a non-human transgenic vertebrate (IV) transfected by (I) or (II) comprising native germ cells carrying in their genomes a xenogenic PN;

(3) a non-human vertebrate (V), carrying in its germ cells a xenogenic PN sequence obtained by breeding (IV) or progeny with a member of the opposite **sex** of the same species, and **selecting** the bred progeny for the presence of the transfected PN;

(4) a germ cell (VI) obtained from (IV) or (V);

(5) vertebrate semen comprising (VI);

(6) a gene therapy method, comprising (I), where PN encoding a desired trait or product is derived from the same species of vertebrate as a recipient vertebrate;

(7) a kit (VII) for genetic alteration and transfer of a male vertebrate's germ cells, containing components of a gene delivery mixture, comprising a transfecting or gene delivery agent, an alkylating agent, and optionally a genetic selection marker, where the kit is used to genetically alter and transfer germ cells in a viable condition;

(8) producing a non-human vertebrate animal line comprising native germ cells carrying in their genome a xenogenic PN, comprising breeding the vertebrate (IV) with a member of the opposite **sex** of the same species and **selecting** progeny for the presence of the PN; and

(9) a method of isolating or selecting a male germ cell transfected with a PN encoding a desired trait or product, comprising (II), where the gene delivery mixture comprises a PN encoding a genetic selection marker and isolating the genetically altered male germ cell with the aid of the genetic selection marker.

ACTIVITY - Antidiabetic; cardiant; hypotensive; neuroleptic.

No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - In vivo and ex vivo transfection of eukaryotic germ cells with a desired genetic material is useful for producing transgenic animals for use as animal models, for producing therapeutic products such as pharmaceuticals in domestic cow's milk and for generating transgenic animals of suitable anatomical and physiological phenotype for human xenograft transplantation. The method is also useful for treating animals, particularly humans, with disorders of **spermatogenesis**.

ADVANTAGE - Depopulating the vertebrate testis maximizes the production of transgenic animals using (I). The transfection method is less costly, faster and more efficient in producing transgenics.

Dwg.0/3

L4 ANSWER 3 OF 6 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1996-238761 [24] WPIDS
DOC. NO. CPI: C1996-076159
TITLE: Apparatus for **sorting** mammalian **sperm** according to **sex** - comprises a column packed with two regions of differently sized beads, acting as a mechanical sieve.
DERWENT CLASS: B07 C07
INVENTOR(S): CHANDLER, J E
PATENT ASSIGNEE(S): (LOUU) UNIV LOUISIANA STATE & AGRIC & MECH COLL
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5514537	A	19960507	(199624)*		8

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 5514537	A	US 1994-347793	19941128

PRIORITY APPLN. INFO: US 1994-347793 19941128
AN 1996-238761 [24] WPIDS
AB US 5514537 A UPAB: 19960618
Appts. for sorting **sperm** into two fractions, each enriched in **sperm** bearing the X or Y chromosome, comprises a column tightly packed in two separate but adjacent regions with spherical beads of uniform size. The second type of beads has a radius (R2) such that a length equal to 0.155 R2 is intermediate between the mean radii of **sperm** carrying the X chromosome and of those carrying the Y chromosome, of a particular mammalian species. The first beads have a radius (R1) larger than R2.
USE - The method is used to fractionate equine, **ovine** or porcine, esp. bovine **sperm**.
ADVANTAGE - **Sperm** are now sorted by a simple sieving system, and remain viable after sorting.
Dwg.0/0

L4 ANSWER 4 OF 6 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 96:878913 SCISEARCH
THE GENUINE ARTICLE: VU392
TITLE: Birth of a male lamb derived from an in vitro matured oocyte fertilised by intracytoplasmic injection of a single presumptive male **sperm**
AUTHOR: Catt S L (Reprint); Catt J W; Gomez M C; Maxwell W M C; Evans G
CORPORATE SOURCE: UNIV SYDNEY, DEPT ANIM SCI, SYDNEY, NSW 2006, AUSTRALIA (Reprint); ST GEORGE HOSP, KOGARAH, NSW 2217, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA
SOURCE: VETERINARY RECORD, (16 NOV 1996) Vol. 139, No. 20, pp. 494-495.
Publisher: BRITISH VETERINARY ASSOC, 7 MANSFIELD ST,

LONDON, ENGLAND W1M 0AT.
ISSN: 0042-4900.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: English
REFERENCE COUNT: 15
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The developmental competence of in vitro matured **ovine** oocytes, cytoplasmically injected with single male or female chromosome-bearing **sperm**, was investigated. Eighty-five unsorted, 92 'female-sorted' and 74 'male-sorted' ram **sperm** were injected into in vitro matured sheep oocytes and, two to four hours later, placed into the oviducts of 28 oestrous sheep, The **sperm** were separated according to sex by analysis of their DNA content with a now cytometer. One pregnancy was diagnosed by ultrasound after 55 days and a 3 kg male lamb was born after 150 days gestation, This lamb was derived from an oocyte injected with 'male-sorted' **sperm**.

L4 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 95:538488 SCISEARCH
THE GENUINE ARTICLE: RM593
TITLE: COMPARATIVE INTRACYTOPLASMIC **SPERM** INJECTION (ICSI) IN HUMAN AND DOMESTIC SPECIES
AUTHOR: CATT J W (Reprint); RHODES S L
CORPORATE SOURCE: ROYAL N SHORE HOSP, N SHORE ART, CLIN 20, ST LEONARDS, NSW 2065, AUSTRALIA (Reprint); UNIV SYDNEY, DEPT ANIM SCI, SYDNEY, NSW 2006, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA
SOURCE: REPRODUCTION FERTILITY AND DEVELOPMENT, (1995) Vol. 7, No. 2, pp. 161-167.
ISSN: 1031-3613.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The current clinical use of intracytoplasmic **sperm** injection (ICSI) for the alleviation of male factor infertility has prompted a re-investigation of **sperm** injection techniques in a number of animal species. This report examines **sperm** injection of in vitro matured oocytes in the major domestic species and compares the results with the human. **Ovine**, bovine and porcine oocytes can undergo fertilization and at least limited development without exogenous activation either prior to or subsequent to injection. Porcine is temperature sensitive during fertilization and the early stages of embryo development. The oocytes of all three domestic species, particularly **ovine**, have a tendency to activate after the injection procedure regardless of the presence or absence of **sperm**. The implications for early development studies and the practical use of direct **sperm** injection for domestic species are discussed.

L4 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 92096714 MEDLINE
DOCUMENT NUMBER: 92096714 PubMed ID: 2132706
TITLE: Reproductive technology in animal production.
AUTHOR: Shelton J N

CORPORATE SOURCE: John Curtin School of Medical Research, Australian National University, Division of Clinical Sciences, Canberra City, ACT.
SOURCE: REVUE SCIENTIFIQUE ET TECHNIQUE, (1990 Sep) 9 (3) 825-45. Ref: 135
Journal code: 8712301. ISSN: 0253-1933.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920223
Last Updated on STN: 19920223
Entered Medline: 19920206

AB Research into physiology and embryology has provided a basis for the development of technologies that increase productivity of farm animals through enhanced control of reproductive function. Progestagens, alone or in combination with luteolysins, are used to control the time of oestrus in cattle, sheep and pigs, thus permitting better use of artificial insemination, providing synchronised recipients for embryos and facilitating management strategies. Treatment with progestagens and pregnant mare serum gonadotrophin (PMSG) or with gonadotrophin releasing hormone induces breeding activity in sheep and goats before the commencement of the breeding season and reduces the duration of postpartum anoestrus in cattle. In pigs, gonadotrophins are used to hasten puberty in gilts, control the time of oestrus in sows and gilts and reduce the interval between farrowing and oestrus. Implants of melatonin hasten the onset of the breeding season in sheep and goats. Success in increasing litter size in sheep and cattle with PMSG has been limited because of the large variation in response between animals. Likewise, immunisation against steroids has not given consistent results. Immunisation against inhibin appears to offer the possibility of increasing farm animal fecundity. Induction of twinning in cattle by embryo transfer is practicable, and recent developments suggest that in vitro fertilisation may provide a source of embryos for this purpose. Real-time ultrasonic scanning has proved to be a reliable method for diagnosing pregnancy in small ruminants and pigs. The identification of pregnancy-specific proteins in cattle and sheep may provide a cheap and practical serological test for pregnancy in these species. Partial segregation of **spermatozoa** into X- and Y-bearing components has been reported, but the method is not yet practicable for use in conventional artificial insemination of farm animals. The sex of bovine and **ovine** embryos can be determined reliably by DNA probes specific for the Y chromosome. Monozygous twins can be produced in all farm animal species by microsurgical bisection of embryos and techniques for cloning from embryonic cells are rapidly being developed. There is a need to devise strategies to utilise these clones to best advantage in genetic programmes. Chimeric animals can be produced in the common farm animal species and will play an important role in genetic engineering, particularly when embryonic stem cell lines are produced in these species.

(d) establishing a sheath fluid which is adapted to form droplets and which is compatible with the ESCs;
(e) establishing a skim milk solution into which the ESCs are collected;
(f) discriminating between the ESCs according to a determination of their sex characteristic;
(g) entraining individual ESCs in a droplet;
(h) sorting the droplets according to the sex of the individual ESCs they contain; and
(i) collecting ESCs having the desired sex characteristic in the skim milk solution;
(2) an equine-adapted flow cytometer system for isolating desired cells comprising:
(a) an ESC source which supplies cells to be analyzed by the flow cytometer;
(b) a chemically coordinated sheath fluid source which creates a sheath fluid environment for the ESCs;
(c) a nozzle through which the ESCs pass while subjected to the sheath fluid environment;
(d) an oscillator which acts upon the sheath fluid as it passes through the nozzle;
(e) a cell sensing system which responds to the ESCs;
(f) a ESC sorter discrimination system which acts to sort the ESCs having a desired sex characteristic; and
(g) a skim milk solution collector into which the ESCs having the desired sex characteristic are placed.
USE - The methods can be used for the artificial insemination of equine mammals to produce equine offspring, particularly offspring of the desired sex (claimed).
ADVANTAGE - The methods can provide low dose, sexed and unsexed non-surgical artificial insemination of equines on a commercial basis.
Dwg.0/2

L6 ANSWER 10 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 2
ACCESSION NUMBER: 2000:361739 SCISEARCH
THE GENUINE ARTICLE: 311TB
TITLE: Modified insemination procedure in **cattle**: deep uterine deposition of smaller numbers of frozen-thawed or **sex-selected spermatozoa**
AUTHOR: Hunter R H F (Reprint)
CORPORATE SOURCE: 32 GILMOUR RD, EDINBURGH EH16 5NT, MIDLOTHIAN, SCOTLAND (Reprint); UNIV CAMBRIDGE, FAC VET MED, CAMBRIDGE, ENGLAND
COUNTRY OF AUTHOR: SCOTLAND; ENGLAND
SOURCE: REVUE DE MEDECINE VETERINAIRE, (MAR 2000) Vol. 151, No. 3, pp. 187-196.
Publisher: ECOLE NATIONAL VET TOULOUSE, 23 CHEMIN DES CAPELLES, 31076 TOULOUSE, FRANCE.
ISSN: 0035-1555.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: AGRI
LANGUAGE: English
REFERENCE COUNT: 68
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB After describing the site of fertilisation and that of the functional sperm reservoir in the female tract, proposals are made

concerning a modified site of sperm deposition. A deep pre-ovulatory insemination into the ipsilateral uterine horn - the side adjoining the ovulatory follicle - should raise the chances of establishing viable spermatozoa in the isthmus where they would undergo storage, Suppressed morility within viscous secretions and binding of the head to endosalpingeal microvilli characterise this phase, Release and activation of such spermatozoa would be prompted by imminent ovulation and associated endocrine programming delivered via a local route.

Potential advantages of deep insemination include (I) raising the overall fertility of genetically valuable bulls whose non-return rates are sub-optimal; (2) reducing the number of spermatozoa in each insemination dose; (3) using effectively the limited numbers of sex-selected sperm cells (X and Y chromosome bearing, spermatozoa), currently available from flow cytometry; and (4) breeding from valuable but oligospermic bulls, Putative disadvantages might include (1) rectal palpation of the ovaries to locate the preovulatory follicle; (2) damage or even perforation of the uterine wall by the;deep insemination catheter; (3)risk of polyspermic fertilisation; and (4) the inappropriateness of the technique for non-clinically qualified inseminators. Each of these reservations is responded to in a rational manner. In conclusion, a modified technique of insemination would be feasible under commercial conditions, might enable retention of genetically valuable bulls deemed of only average fertility under test conditions, and could give a welcome boost to a sagging artificial insemination industry.

L6 ANSWER 11 OF 41 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000402683 MEDLINE
DOCUMENT NUMBER: 20305226 PubMed ID: 10844187
TITLE: Sexing mammalian sperm for production of offspring: the state-of-the-art.
AUTHOR: Johnson L A
CORPORATE SOURCE: Germplasm and Gamete Physiology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, USA.. lajohnson@lpsi.barc.usda.gov
SOURCE: ANIMAL REPRODUCTION SCIENCE, (2000 Jul 2) 60-61 93-107. Ref: 47
Journal code: 7807205. ISSN: 0378-4320.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000901
Last Updated on STN: 20000901
Entered Medline: 20000822

AB Predetermination of sex in livestock offspring is in great demand and is of critical importance to providing for the most efficient production of the world's food supply. With the changes that have taken place in animal agriculture over the past generation the application of sex preselection to production systems becomes increasingly necessary. The current technology is based on the well-known difference in X- and Y-sperm in the amount of DNA present. The method has been validated on the basis of live births,

laboratory reanalysis of sorted sperm for DNA content and embryo biopsy for sex determination. The technology incorporates modified flow cytometric sorting instrumentation to sort X- and Y-bearing sperm. Resulting populations of X or Y sperm can be used in conjunction with IVF in swine and in **cattle** for the production of sexed embryos to be transferred to eligible recipients for the duration of gestation. It can also be used for intratubal insemination and for deep-uterine and conventional insemination in **cattle**. This semipractical sexing method, though currently impractical for some production systems (where large numbers of sperm are required for fertilization) could be used to provide a more flexible progeny-producing option in many livestock operations. Improvements in the production rate of sexed sperm continue as new technology is developed. High-speed sorting is one of the newer technological advances and is being used in our laboratory to increase sorted sperm throughput. With our original technology we sorted 350,000 **sperm**/h. We now **sort** 6 million of each **sex**, under routine conditions. Sorting only the X population results in about 18 million sperm/h. Improvements in the technology will no doubt lead to much greater usage of sexed sperm, depending on the species involved. Insemination of lower sperm numbers in **cattle** has proven to be an effective means of utilizing the sexing technology. Solving the problems associated with inseminating low sperm numbers in the pig would be advantageous to the utilization of sexed sperm for some type of deep artificial insemination. Such a development would also enhance the economy of using lower sperm numbers with conventional artificial insemination (AI) and aid the swine industry worldwide. The use of sexed sperm for non-ordinary applications such as endangered species, laboratory animals, hobby or pet species is also of interest and will become a part of the move to be more reproductively efficient in the next millennium. Sexed sperm on demand over the next several years will provide livestock producers with many options in seeking to improve efficiency of production and improve quality of products to enhance consumer acceptability.

L6 ANSWER 12 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1999-493811 [41] WPIDS
CROSS REFERENCE: 2000-182914 [16]; 2002-098005 [13]; 2002-681828 [73]
DOC. NO. NON-CPI: N1999-367845
DOC. NO. CPI: C1999-144655
TITLE: Sex-specific insemination of mammals with low number of sperm count.
DERWENT CLASS: B04 C06 D16 P14 P32
INVENTOR(S): HERICKHOFF, L; SCHENK, J; SEIDEL, G E; SEIDEL, G
PATENT ASSIGNEE(S): (COLS) UNIV COLORADO STATE; (XYXY-N) XY INC; (COLS) UNIV COLORADO STATE RES FOUND
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9933956	A1	19990708	(199941)*	EN	73

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT

LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL
TJ TM TR TT UA UG US UZ VN YU ZW

AU 9920239 A 19990719 (199951)
US 6071689 A 20000606 (200033)
EP 1044262 A1 20001018 (200053) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
BR 9814568 A 20001010 (200055)
NO 2000003424 A 20000830 (200056)
GB 2350619 A 20001206 (200065)
US 6149867 A 20001121 (200101)
DE 19882943 T 20010201 (200108)
CN 1284128 A 20010214 (200130)
HU 2001000286 A2 20010528 (200140)
KR 2001033818 A 20010425 (200164)
ES 2161656 A1 20011201 (200205)
JP 2002500006 W 20020108 (200206) 81
US 6372422 B1 20020416 (200232)
US 2002119558 A1 20020829 (200259)
ES 2161656 B1 20020801 (200263)
MX 2000006526 A1 20011001 (200274)
JP 2002262715 A 20020917 (200276) 28

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9933956	A1		WO 1998-US27909	19981231
AU 9920239	A		AU 1999-20239	19981231
US 6071689	A	CIP of	US 1997-1394	19971231
			US 1998-15454	19980129
EP 1044262	A1		EP 1998-965046	19981231
			WO 1998-US27909	19981231
BR 9814568	A		BR 1998-14568	19981231
			WO 1998-US27909	19981231
NO 2000003424	A		WO 1998-US27909	19981231
			NO 2000-3424	20000630
GB 2350619	A		WO 1998-US27909	19981231
			GB 2000-16132	20000703
US 6149867	A		US 1997-1394	19971231
DE 19882943	T		DE 1998-19882943	19981231
			WO 1998-US27909	19981231
CN 1284128	A		CN 1998-813255	19981231
HU 2001000286	A2		WO 1998-US27909	19981231
			HU 2001-286	19981231
KR 2001033818	A		KR 2000-707374	20000630
ES 2161656	A1		ES 2000-50052	19981231
JP 2002500006	W		WO 1998-US27909	19981231
			JP 2000-526614	19981231
US 6372422	B1	CIP of	US 1997-1394	19971231
		Cont of	US 1998-15454	19980129
			US 1999-448643	19991124
US 2002119558	A1	CIP of	US 1997-1394	19971231
		Cont of	US 1998-15454	19980129
		Cont of	US 1999-448643	19991124
			US 2002-81955	20020220
ES 2161656	B1		ES 2000-50052	19981231
MX 2000006526	A1		MX 2000-6526	20000630
JP 2002262715	A	Div ex	JP 2000-526614	19981231

JP 2002-44035 19981231

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9920239	A	Based on	WO 9933956
EP 1044262	A1	Based on	WO 9933956
BR 9814568	A	Based on	WO 9933956
GB 2350619	A	Based on	WO 9933956
DE 19882943	T	Based on	WO 9933956
HU 2001000286	A2	Based on	WO 9933956
JP 2002500006	W	Based on	WO 9933956
US 6372422	B1	Cont of	US 6071689
		CIP of	US 6149867
US 2002119558	A1	Cont of	US 6071689
		CIP of	US 6149867
		Cont of	US 6372422

PRIORITY APPLN. INFO: US 1998-15454 19980129; US 1997-1394 19971231; US 1999-448643 19991124; US 2002-81955 20020220

AN 1999-493811 [41] WPIDS

CR 2000-182914 [16]; 2002-098005 [13]; 2002-681828 [73]

AB WO 9933956 A UPAB: 20021125

NOVELTY - A method of producing a mammal with a predetermined sex is new.

DETAILED DESCRIPTION - A method for producing a mammal of predetermined sex comprises:

(a) collecting sperm cells from a male species of a mammal;

(b) determining the sex characteristic of the sperm cells;

(c) sorting the sperm cells according to the determination of their sex characteristic;

(d) establishing an insemination sample having a low number of the sperm cells relative to the typical artificial insemination dosage;

(e) inserting at least a portion of the insemination sample into a female species of the mammal;

(f) fertilizing at least one egg within the female at success levels statistically comparable to the typical unsexed artificial insemination dosage; and

(g) producing an offspring mammal of the desired sex.

An INDEPENDENT CLAIM is also provided for a flow cytometer system for isolating desired cells comprising:

(a) a cell source supplying cells to the flow cytometer;

(b) a sheath fluid source creating a sheath fluid environment containing about 2.9 % sodium citrate;

(c) a nozzle through which the cells pass;

(d) an oscillator acting on the sheath fluid;

(e) a cell sensing system which responds to the cells;

(f) a sorter discrimination system which sorts cells having a desired characteristic; and

(g) a collector into which the desired cells are placed.

USE - The sex-specific artificial inseminations especially useful in the breeding of bovine and equine life stock (all claimed).

ADVANTAGE - The success levels of the sex-specific artificial insemination is statistically comparable to the typical artificial

insemination at least 35-90%. The insemination sample required is only at most 10% of the typical number of sperm provided in a typical, unsexed artificial insemination event, e.g. bovine sperm cells 100000-300000 bovine sperm cells or 1-25 million equine sperm cells.
Dwg.0/4

L6 ANSWER 13 OF 41 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 2001:96270 CABA
DOCUMENT NUMBER: 20013091846
TITLE: Current status of sexing mammalian sperm by sperm sorting
AUTHOR: Johnson, L. A.; Russo, V. [EDITOR]; Dall'Olio, S. [EDITOR]; Fontanesi, L. [EDITOR]
CORPORATE SOURCE: Germplasm and Gamete Physiology Laboratory, Agricultural Research Service, US Department of Agriculture, Beltsville, Maryland, USA.
SOURCE: Proceedings of the International Symposium, Reggio Emilia, Italy, 8-9 October, 1999. From the first artificial insemination to the modern reproduction biotechnologies: traditional ways and new frontiers of animal production, (1999) pp. 105-111. 21 ref.
Publisher: University of Bologna. Reggio Emilia
Meeting Info.: Proceedings of the International Symposium, Reggio Emilia, Italy, 8-9 October, 1999. From the first artificial insemination to the modern reproduction biotechnologies: traditional ways and new frontiers of animal production..
PUB. COUNTRY: Italy
DOCUMENT TYPE: Book; Book Article; Conference Article
LANGUAGE: English
AB The current status of sexing **mammalian sperm** by **sperm sorting** is discussed. **Sex preselection** is a tool in improving the livestock production efficiency, and the technology works well in virtually all domestic animal species with at least 90% accuracy rate of production of the correct sex.

L6 ANSWER 14 OF 41 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2000199263 MEDLINE
DOCUMENT NUMBER: 20199263 PubMed ID: 10735088
TITLE: Issues affecting commercialization of sexed sperm.
AUTHOR: Amann R P
CORPORATE SOURCE: BioPore Inc., State College, PA 16805-0074, USA.
SOURCE: THERIOGENOLOGY, (1999 Dec) 52 (8) 1441-57. Ref: 29
Journal code: 0421510. ISSN: 0093-691X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505

Searcher : Shears 308-4994

Entered Medline: 20000426

AB A decision tree for genetics or sperm-sexing entities considering sales of sexed sperm is discussed in terms of: (a) how best to avoid harm; (b) how best to do good; (c) needed synergy with other assisted reproductive technologies; (d) constraints on biotechnology; and (e) costs with current and likely technologies versus potential benefits to producers. The sexed-sperm industry might wish to take a pro-active stance on societal issues potentially affecting use of sexed sperm. For most sales in animal agriculture, cost of added value must be < 50% of benefit. Cost is less important for emotionally-driven uses with **horses** and human beings. Current procedures for flow-sorting allow most sperm to retain their fertilizing potential. Added cost to produce and package 2 x 10(6) sperm is estimated at US $30 to US $46 with flow sorted sperm. Estimating cost of any alternative technology is premature. For IVF/embryo transfer (ET), cost and numbers of flow-**sorted sexed sperm** are appropriate for commercial use. For use in low-dose AI, however, added cost to supply one insemination dose must be near US $12. Flow-sorting instruments with higher throughput and lower purchase and operating costs are obligatory for economic application in most AI situations. Developers of antibody-based separations also will face issues of retention of fertilizing potential while minimizing cell loss, separation of living from dead sperm concurrent with sperm sexing, output, and cost. To benefit producers and consumers in a changing world, genetics and sperm-sexing companies will have to collaborate and interface to provide funding for needed research and development and to recover these costs, using mechanisms not yet obvious.

L6 ANSWER 15 OF 41 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 2000199259 MEDLINE
DOCUMENT NUMBER: 20199259 PubMed ID: 10735084
TITLE: In vitro fertilization with flow-cytometrically-sorted bovine sperm.
AUTHOR: Lu K H; Cran D G; Seidel G E Jr
CORPORATE SOURCE: XY, Inc., Fort Collins, CO 80523, USA.
SOURCE: THERIOGENOLOGY, (1999 Dec) 52 (8) 1393-405.
Journal code: 0421510. ISSN: 0093-691X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505
Entered Medline: 20000426

AB An attractive feature of IVF is that fewer sexed sperm are needed than for artificial insemination. However, **sperm sexed** by flow cytometry/cell **sorting** are probably pre-capacitated, necessitating modifications to standard IVF systems for optimal success. With current procedures, the percentages of oocytes fertilized with sorted and unsorted frozen **bovine** sperm are similar, and events during the first cell cycle are timed similarly for sorted and unsorted sperm. However, in most cases, blastocyst production with sorted sperm was approximately 70% of controls produced with unsorted sperm. In some early studies, there appeared to be an unexplained delay of about half a day in blastocyst development. Nevertheless, some dozens of apparently

normal calves, pre-sexed with 90% accuracy, have resulted from frozen embryos produced via IVF with sexed sperm. IVF also has proven useful as a bioassay for improving sperm-sorting procedures such as determining potential detrimental effects of laser power. It is likely that use of IVF in **cattle** breeding programs will increase considerably when sexed, frozen sperm become commercially available.

L6 ANSWER 16 OF 41 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2000199254 MEDLINE
DOCUMENT NUMBER: 20199254 PubMed ID: 10735079
TITLE: Sex preselection: high-speed flow cytometric sorting of X and Y sperm for maximum efficiency.
AUTHOR: Johnson L A; Welch G R
CORPORATE SOURCE: Germplasm and Gamete Physiology Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, USA.
SOURCE: THERIOGENOLOGY, (1999 Dec) 52 (8) 1323-41. Ref: 49
Journal code: 0421510. ISSN: 0093-691X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505
Entered Medline: 20000426

AB Sex preselection that is based on flow-cytometric measurement of sperm DNA content to enable sorting of X- from Y-chromosome-bearing sperm has proven reproducible at various locations and with many species at greater than 90% purity. Offspring of the predetermined sex in both domestic animals and human beings have been born using this technology since its introduction in 1989. The method involves treating sperm with the fluorescent dye, Hoechst 33342, which binds to the DNA and then sorting them into X- and Y-bearing-sperm populations with a flow cytometer/cell **sorter** modified specifically for **sperm. Sexed sperm** are then used with differing semen delivery routes such as intra-uterine, intra-tubal, artificial insemination (deep-uterine and cervical), in vitro fertilization and embryo transfer, and intra-cytoplasmic sperm injection (ICSI). Offspring produced at all locations using the technology have been morphologically normal and reproductively capable in succeeding generations. With the advent of high-speed cell sorting technology and improved efficiency of sorting by a new sperm orienting nozzle, the efficiency of sexed sperm production is significantly enhanced. This paper describes development of the these technological improvements in the Beltsville Sexing Technology that has brought sexed sperm to a new level of application. Under typical conditions the high-speed sperm sorter with the orienting nozzle (HiSON) results in purities of 90% of X- and Y-bearing sperm at 6 million sperm per h for each population. Taken to its highest performance level, the HiSON has produced X-bearing-sperm populations at 85 to 90% purity in the production of up to 11 million X-bearing-sperm per h of sorting. In addition if one accepts a lower purity (75 to 80%) of X, nearly 20 million sperm can be sorted per h. The latter represents a 30 to 60-fold improvement over the 1989 sorting technology using rabbit

sperm. It is anticipated that with instrument refinements the production capacity can be improved even further. The application of the current technology has led to much wider potential for practical usage through conventional and deep-uterine artificial insemination of many species, especially **cattle**. It also opens the possibility of utilizing sexed sperm for artificial insemination in swine once low-sperm-dose methods are perfected. Sexed sperm on demand has become a reality through the development of the HiSON system.

L6 ANSWER 17 OF 41 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000199250 MEDLINE
DOCUMENT NUMBER: 20199250 PubMed ID: 10735075
TITLE: Effect of timing of artificial insemination on sex ratio.
AUTHOR: Rorie R W
CORPORATE SOURCE: Animal Science Department, University of Arkansas, Fayetteville, USA.
SOURCE: THERIOGENOLOGY, (1999 Dec) 52 (8) 1273-80. Ref: 30
Journal code: 0421510. ISSN: 0093-691X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505
Entered Medline: 20000426

AB For a number of years, the time of insemination or mating during estrus has been believed to influence the sex ratio of offspring, with early insemination resulting in more females and late insemination, more males. Possible mechanisms of altering the sex ratio include facilitating or inhibiting the transport of either X- or Y-chromosome-bearing **sperm** through the reproductive tract, preferential **selection** of **sperm** at fertilization, or **sex**-specific death of embryos after fertilization. In livestock species, there is evidence for preferential selection of X- or Y-bearing sperm, based on the maturational state of the oocyte at fertilization. In deer and **sheep**, early and late insemination appears to skew the sex ratio toward females and males, respectively. In **cattle**, conflicting reports on the effect of time of insemination on sex ratio make the premise less clear. Many of the published studies lack adequate observations for definitive conclusions and/or are based on infrequent observations of estrus, making it difficult to assess the effect of time of insemination on sex ratio. It is likely that any effect of time of insemination on sex ratio in **cattle** is relatively small. Evidence is accumulating that treatments used for synchronization of estrus or ovulation in **cattle** may influence the sex ratio.

L6 ANSWER 18 OF 41 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1999283989 MEDLINE
DOCUMENT NUMBER: 99283989 PubMed ID: 10357097
TITLE: Mammalian Y chromosome evolution and the male-specific functions of Y chromosome-borne genes.

AUTHOR: Delbridge M L; Graves J A
CORPORATE SOURCE: Department of Biochemistry and Genetics, La Trobe University, Bundoora, Victoria, Australia.
SOURCE: REVIEWS OF REPRODUCTION, (1999 May) 4 (2) 101-9.
Ref: 63
Journal code: 9602351. ISSN: 1359-6004.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990730
Last Updated on STN: 19990730
Entered Medline: 19990722

AB All **mammals** have an XY chromosomal sex determining system, in which a small Y chromosome triggers male development, and contains genes required for spermatogenesis. The X and Y chromosomes were originally homologous, but diverged during evolution as the Y chromosome was degraded progressively. Comparisons among the sex chromosomes of different **mammal** groups indicate that the X and Y chromosomes received additions of material from other chromosomes. Genes on the Y chromosome originated from the ancient X-Y pair, or from these additions, or were copies of genes on one of the autosomes. Only genes with important male-specific functions, such as **sex** determination and **spermatogenesis**, are **selected** for and retained on the differential region of the Y chromosome. The **mammalian** sex determining gene, SRY, controls the testis determination pathway, which includes at least one related gene. Several candidate spermatogenesis genes have been identified, but so far the only one that is conserved on the Y chromosome of all therian **mammals** is RBM (RNA-binding motif gene, Y chromosome).

L6 ANSWER 19 OF 41 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1999003747 MEDLINE
DOCUMENT NUMBER: 99003747 PubMed ID: 9787479
TITLE: Deep uterine insemination of cattle: a fruitful way forward with smaller numbers of spermatozoa.
AUTHOR: Hunter R H; Greve T
CORPORATE SOURCE: Department of Clinical Studies, Reproduction, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.. TG@kvl.dk
SOURCE: ACTA VETERINARIA SCANDINAVICA, (1998) 39 (2) 149-63.
Ref: 64
Journal code: 0370400. ISSN: 0044-605X.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981120

AB After describing the site of fertilisation and that of the

functional sperm reservoir in the female tract, proposals are made concerning a modified site of sperm deposition in **cattle**. By means of a deep pre-ovulatory insemination into the ipsilateral uterine horn, the chances should be raised of establishing viable spermatozoa in the isthmus where they would undergo a form of physiological encapsulation and storage. Release and activation of such spermatozoa would be prompted by imminent ovulation. Potential advantages of this approach include those of raising the overall fertility of genetically valuable bulls whose non-return rates are sub-optimal; reducing the number of spermatozoa in each insemination dose; using effectively the limited numbers of **sex-selected sperm** cells (X and Y chromosome bearing spermatozoa) currently available from flow cytometry. Putative disadvantages might include rectal palpation of the ovaries to locate the pre-ovulatory follicle; perforation of the uterine wall by the deep insemination catheter; risk of polyspermic fertilisation; and the inappropriateness of the technique for non-clinically qualified inseminators. Each of these reservations is responded to in a rational manner. Given a change of attitude, a modified technique of insemination would be feasible under commercial conditions and might give a welcome boost to a sagging artificial insemination industry.

L6 ANSWER 20 OF 41 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 1998353785 MEDLINE
DOCUMENT NUMBER: 98353785 PubMed ID: 9689360
TITLE: H-Y antigen expression patterns in human X- and Y-chromosome-bearing spermatozoa.
AUTHOR: Sills E S; Kirman I; Colombero L T; Hariprashad J; Rosenwaks Z; Palermo G D
CORPORATE SOURCE: Department of Obstetrics and Gynecology, New York Hospital-Cornell Medical Center, New York 10021, USA.
SOURCE: AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY, (1998 Jul) 40 (1) 43-7.
Journal code: 8912860. ISSN: 1046-7408.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981021
Last Updated on STN: 19981021
Entered Medline: 19981015

AB PROBLEM: Restricted expression of H-Y antigen on Y-chromosome-bearing sperm has been reported in some species, although such preferential expression for H-Y antigen in human sperm has yet to be described. In this study, an immunomagnetic approach was used to characterize antigen expression patterns as a function of sex-chromosome content. METHOD OF STUDY: Human sperm was treated with monoclonal immunoglobulin (Ig) M antibodies directed against H-Y antigen. This preparation then was incubated with **sheep** antimouse IgM antibody affixed to paramagnetic beads, which then were exposed to a magnetic field and sorted. X- and Y-chromosome frequencies in the two subgroups of sperm were assayed by multiprobe fluorescent in situ hybridization (FISH). RESULTS: Sperm were immunomagnetically separated into two populations: a reactive group (presumably, H-Y Ag+); and a nonreactive group (presumably, H-Y Ag-). Triple-color FISH analysis of 1,600 spermatozoa (800 in each

group) showed the antigen's expression to be somewhat more prevalent among Y-chromosome-bearing sperm (54.1%), but a large proportion of Y-chromosome-bearing sperm (49.0%) did not express this antigen. The difference was not significant (P = 0.43). CONCLUSIONS: The expression of H-Y antigen has a slightly higher frequency in human sperm containing the Y-chromosome, but its expression among X-chromosome-bearing sperm also is considerable. Current immunologic techniques relying on this antigen are unlikely to effect the **sex selection** of human **sperm**.

L6 ANSWER 21 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 11
ACCESSION NUMBER: 1998:432953 BIOSIS
DOCUMENT NUMBER: PREV199800432953
TITLE: Recent improvements in efficiency of flow cytometric sorting of X and Y-chromosome bearing sperm of domestic animals: A review.
AUTHOR(S): Gurnsey, M. P. (1); Johnson, L. A.
CORPORATE SOURCE: (1) AgRes., Dairy and Beef Div., Ruakura Agric. Res. Cent., Private Bag 3123, Hamilton New Zealand
SOURCE: Proceedings of the New Zealand Society of Animal Production, (1998) Vol. 58, No. 0, pp. 16-18. ISSN: 0370-2731.
DOCUMENT TYPE: Article
LANGUAGE: English
AB In **mammals** the only method known to reliably separate X and Y sperm for producing offspring of a specific sex is flow cytometric sorting. This method is based on the observation that X-chromosome bearing sperm of domestic animals contain 3.5-4.2% more DNA than Y-chromosome bearing sperm. Relative DNA content is determined by quantitative staining with Hoechst 33342 and DNA content measured using a modified cell sorter. The major constraint to widespread application of this technology has been the slow sorting rate. Using modified standard speed cell sorters, sample flow rates average 2,000 sperm/sec with 25-35% orientation. Recently, a high speed sorter (MoFlo) modified for sperm sorting has been used and increases sorting rate by approximately 5-fold. Further, an orienting nozzle has been developed which increases the percentage of sperm that are orientated thus increasing the sort rate by a further 2 to 3-fold. Combined, these improvements have increased the production of sorted sperm from approximately 0.3 X 106/h with conventional sorters to at least 4 X 106/h with the high speed sorter. Preliminary testing suggests that purity of sort and sperm viability are not compromised by increased sorting rates. Pregnancies have been established in pigs and **cows** with sorted sperm from a modified MoFlo fitted with a novel nozzle. The improvements outlined here greatly enhance the Beltsville Sperm Sexing Technology and make it realistic to consider trials using **sex sorted sperm** for conventional AI as well as deep uterine AI in **cattle**.

L6 ANSWER 22 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1996-238761 [24] WPIDS
DOC. NO. CPI: C1996-076159
TITLE: Apparatus for **sorting mammalian sperm** according to **sex** - comprises a column packed with two regions of differently sized beads, acting as a mechanical

sieve.
DERWENT CLASS: B07 C07
INVENTOR(S): CHANDLER, J E
PATENT ASSIGNEE(S): (LOUU) UNIV LOUISIANA STATE & AGRIC & MECH COLL
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5514537	A	19960507	(199624)*		8

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 5514537	A	US 1994-347793	19941128

PRIORITY APPLN. INFO: US 1994-347793 19941128
AN 1996-238761 [24] WPIDS
AB US 5514537 A UPAB: 19960618
Appts. for sorting sperm into two fractions, each enriched in sperm bearing the X or Y chromosome, comprises a column tightly packed in two separate but adjacent regions with spherical beads of uniform size. The second type of beads has a radius (R2) such that a length equal to 0.155 R2 is intermediate between the mean radii of sperm carrying the X chromosome and of those carrying the Y chromosome, of a particular mammalian species. The first beads have a radius (R1) larger than R2.
USE - The method is used to fractionate equine, ovine or porcine, esp. bovine sperm.
ADVANTAGE - Sperm are now sorted by a simple sieving system, and remain viable after sorting.
Dwg.0/0

L6 ANSWER 23 OF 41 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 96438134 MEDLINE
DOCUMENT NUMBER: 96438134 PubMed ID: 8840588
TITLE: Gender preselection in mammals: an overview.
AUTHOR: Johnson L A
CORPORATE SOURCE: Germplasm and Gamete Physiology Laboratory, Agricultural Research Service U. S. Department of Agriculture, Beltsville, Maryland, USA.
SOURCE: DTW. DEUTSCHE TIERARZTLICHE WOCHENSCHRIFT, (1996 Aug-Sep) 103 (8-9) 288-91. Ref: 19
Journal code: 7706565. ISSN: 0341-6593.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970203
AB Recent advances in the separation of X and Y chromosome bearing spermatozoa have led to the availability of a method (Beltsville

Sperm Sexing Technology) to **preselect** the **sex** in several **mammals**. Progeny using this procedure have been produced in **cattle**, **sheep**, swine and laboratory animals. **Mammalian** sperm are inherently different in that the X sperm carries from 2.8 to 7.5% more DNA than the Y sperm. Individual sperm DNA can be determined and used as the differentiating characteristic with flow cytometry and cell sorting instrumentation especially modified to measure small amounts of DNA in sperm. The process utilizes the fluorochrome Hoechst 33342 to bind to the DNA. The relative DNA is measured by passing the living sperm through a laser beam and collecting the light energy from the individual sperm. Data is acquired and used to select the particular sperm for deflection into collection tubes. The proportions of sorted X and Y sperm in each tube can be validated by reanalyzing an aliquot for DNA content. This value is then used to predict the outcome of fertilization and subsequent gestation. The sorted sperm are used to inseminate eggs via in vitro fertilization (IVF) or by surgical insemination into the oviduct or the uterus of appropriate females. Sperm are sorted at the rate of 0.5 million per hour for most species with the expectation of 90% or greater of one sex or the other being born. Progeny in **cattle** using IVF have been produced at greater than 90% accuracy. Rabbits have produced greater than 90% females using this process. Progeny produced from pigs average 85% for one sex or the other. All progeny produced (N = or > 300) have exhibited completely normal morphological appearance and normal reproductive function. Because of the inability to obtain large numbers of sorted sperm in a short amount of time, the technologies use for regular artificial insemination would not be practical in most domestic species. This sexing technology however is very applicable where IVF, intrauterine or intratubal insemination are convenient means for producing offspring. In addition, the recent advent of ultrasound guided insemination in **cattle** may provide and opportunity to use this technology for much lower numbers of sperm per insemination than previously thought possible. Using less than 2 x 10 sorted X or Y sperm would move the technology one step closer to practicality.

L6 ANSWER 24 OF 41 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 1998:59049 CABA
DOCUMENT NUMBER: 982204288
TITLE: **Preselection** of the **sex** of **cattle** by layering **spermatozoa** on protein columns
AUTHOR: Uavechanichkul, R.; Sukhato, P.; Ratanapaskorn, M.
CORPORATE SOURCE: Artificial Insemination Division, Department of Livestock Development, Phyathai, Bangkok 10400, Thailand.
SOURCE: Journal of the Thai Veterinary Medical Association, (1996) Vol. 47, No. 2, pp. 55-62. 33 ref.
DOCUMENT TYPE: Journal
LANGUAGE: Thai
SUMMARY LANGUAGE: English
AB Diluted semen from AI bulls was layered on a column of bovine serum albumin (BSA) allowing the spermatozoa to swim into it. Semen was recovered, frozen, and used to inseminate 465 cows. Spermatozoa from the top of the BSA column produced 33 (57.9%) male and 24 (42.1%)

female calves, while spermatozoa from the bottom of the column produced 30 (54.5%) male and 25 (45.5%) female calves. The numbers of males and females as well as the conception rates of the semen from the top and the bottom of the columns did not differ significantly.

L6 ANSWER 25 OF 41 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 96:29148 CABA
DOCUMENT NUMBER: 960100961
TITLE: Sex preselection by flow cytometric separation of X and Y chromosome-bearing sperm based on DNA difference: a review
AUTHOR: Johnson, L. A.
CORPORATE SOURCE: Germplasm & Gamete Physiology Laboratory, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, USA.
SOURCE: Reproduction, Fertility and Development, (1995) Vol. 7, No. 4, pp. 893-903. 46 ref. ISSN: 1031-3613
DOCUMENT TYPE: Journal
LANGUAGE: English
AB XI- and Y-chromosome-bearing spermatozoa can be separated by flow cytometry. The spermatozoa are exposed before sorting to the vital dye Hoechst 33342, which binds to the minor groove of the DNA helix. Flow cytometric sorting using a laser as the excitation source results in populations of X- and Y-bearing spermatozoa that are 85-90% pure. Several hundred offspring that confirm the predicted **sex** after fertilization by flow-**sorted** **spermatozoa** have been produced from pigs, rabbits, **sheep** and **cattle**. The method is currently being applied to the commercial embryo market. The method in its current form is not likely to be used in conjunction with standard **cattle** and pig AI since only approximately 4 x 105 sorted spermatozoa can be produced per hour of sorting.

L6 ANSWER 26 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 94:550659 SCISEARCH
THE GENUINE ARTICLE: PE614
TITLE: COMMERCIAL APPLICATIONS OF IN-VITRO FERTILIZATION IN CATTLE
AUTHOR: HASLER J F (Reprint)
CORPORATE SOURCE: EM TRAN INC, ELIZABETHTOWN, PA, 00000 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: COMPENDIUM ON CONTINUING EDUCATION FOR THE PRACTICING VETERINARIAN, (AUG 1994) Vol. 16, No. 8, pp. 1062-1073.
ISSN: 0193-1903.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: No References Keyed
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The historical development and commercial applications of in vitro techniques in **cattle** are reviewed in this article, and relevant data from the commercial in vitro program at a private company are presented. In 1959, the rabbit was the first **mammalian** species in which live offspring were produced by in vitro fertilization. The next success was with laboratory mice

in 1968, because mouse oocysts are inexpensive and can be readily used in multifactorial experiments. The progress noted in mice, however, did not prove to be a good model for developing in vitro procedures in **cattle**. The efficiency of in vitro maturation, fertilization, and culture procedures has improved, paving the way for extensive research using immature oocytes obtained from ovaries procured from slaughterhouses as starting material. Some of the future applications of in vitro technology are fertilization with **sex-selected sperm**, in vitro fertilization of oocytes harvested from fetuses, and genetic analysis of embryos by polymerase chain reaction.

L6 ANSWER 27 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 13
ACCESSION NUMBER: 94:5614 SCISEARCH
THE GENUINE ARTICLE: MM739
TITLE: RECENT ADVANCES IN SEX PRESELECTION OF CATTLE - FLOW CYTOMETRIC SORTING OF X-CHROMOSOME-AND Y-CHROMOSOME BEARING SPERM BASED ON DNA TO PRODUCE PROGENY
AUTHOR: JOHNSON L A (Reprint); CRAN D G; POLGE C
CORPORATE SOURCE: USDA ARS, BELTSVILLE AGR RES CTR, GERMPLASM & GAMETE PHYSIOL LAB, BELTSVILLE, MD, 20705 (Reprint); ANIM BIOTECHNOL CAMBRIDGE LTD, ANIM RES STN, CAMBRIDGE CB3 0JQ, ENGLAND
COUNTRY OF AUTHOR: USA; ENGLAND
SOURCE: THERIOGENOLOGY, (JAN 1994) Vol. 41, No. 1, pp. 51-56

ISSN: 0093-691X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 18
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recent progress, briefly reviewed here has led to the availability of a method of gender preselection in farm animals that can be used for producing progeny in **cattle, sheep**, and swine under semi-practical conditions. Sperm are separated based on the inherent difference in DNA content in the X-and Y-chromosome bearing sperm using flow cytometry/cell sorting technology. Sperm are stained with Hoechst 33342 which binds to the DNA helix in an amount proportional to the amount of DNA thus forming the basis for the method. Calves of predicted **sex** have been born using **sorted sperm** in conjunction with IVF resulting in embryos for transfer. Swine, rabbits and **sheep** have been produced using surgical insemination with smaller numbers of sperm than are required for artificial insemination. The inability to accrue large numbers of sperm in a short period of time precludes standard insemination techniques with sorted sperm. All offspring that have been born using this technology have been morphologically normal, and swine and rabbit offspring have shown normal reproductive function through two generations. Research to streamline hardware and improve staining technology is ongoing, while at the same time the method is being developed for the commercial embryo market.

L6 ANSWER 28 OF 41 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 95:205247 CABA
DOCUMENT NUMBER: 950110830

TITLE: Reproductive management and compact calving in the dairy herd
AUTHOR: Ryan, D. P.; Mee, J. F.
CORPORATE SOURCE: Teagsac, Moorepark Research Centre, Fermoy, Co. Cork, Irish Republic.
SOURCE: Irish Grassland and Animal Production Association Journal, (1994) Vol. 28, pp. 9-18. 33 ref.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effects of management, health, AI techniques, semen quality and mineral deficiencies on infertility in dairy **cows** are discussed. Results of research in the Irish Republic, mainly previously published, on methods for improving reproductive efficiency by better oestrus detection, oestrus synchronization by hormone treatment and improved nutrition are summarized. Studies on **sex selection** by embryo sexing, **sperm sorting** and the effects of the immune system on pregnancy are also presented.

L6 ANSWER 29 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 14
ACCESSION NUMBER: 1994:55500 BIOSIS
DOCUMENT NUMBER: PREV199497068500
TITLE: Fractionation of **bovine spermatozoa** for **sex selection**: A rapid immunomagnetic technique to remove spermatozoa that contain the H-Y antigen.
AUTHOR(S): Peter, A. T. (1); Jones, P. P. (1); Robinson, J. P.
CORPORATE SOURCE: (1) Dep. Veterinary Clin. Sci., Sch. Veterinary Med., Purdue Univ., West Lafayette, IN 47907 USA
SOURCE: Theriogenology, (1993) Vol. 40, No. 6, pp. 1177-1185. ISSN: 0093-691X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A study was conducted to rapidly fractionate bovine spermatozoa on the basis of cell-surface H-Y antigen (i.e., Y chromosome-bearing spermatozoa). A novel, rapid immunomagnetic method was developed for removal of spermatozoa that bound to anti-H-Y IgG. Fluorescent labeling and flow cytometry were used to measure the efficiency with which spermatozoa binding to anti-H-Y were removed by the immunomagnetic technique. Washed bovine spermatozoa (n=7 bulls) were treated with a mouse monoclonal IgG antibody to H-Y antigen (MoAb12/49). Fluorescent labeled goat antibody against mouse IgG was added to label those spermatozoa with cell-surface H-Y antigens. Supermagnetized polymer beads coated with an anti-antibody to the MoAb 12/49 were then added to the spermatozoa. After 20 min of incubation, spermatozoa were exposed for 2 min to a magnet, causing the magnetized particles to adhere to the sides of the tube. Nonmagnetized spermatozoa in the supernatent were aspirated and analyzed for fluorescent label by flow cytometry. Approximately 50% of spermatozoa not subjected to immunomagnetic separation were fluorescent labeled, and about one-half of the spermatozoa were observed microscopically to be bound to the magnetized polymer beads prior to magnetic separation (P lt 0.05). Following magnetic separation, only 1.2% (P lt 0.05) of the spermatozoa in the magnetic supernatent were fluorescent labeled. Assuming that only Y chromosome-bearing spermatozoa have cell-surface H-Y antigens, the

present immunomagnetic fractionation removed almost all of the Y chromosome-bearing spermatozoa, leaving a population that was greater than 98% X chromosome-bearing spermatozoa.

L6 ANSWER 30 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1992:449810 BIOSIS
DOCUMENT NUMBER: BR43:82810
TITLE: INFLUENCE OF FLOW CYTOMETRIC SORTING ON SPERM MEMBRANE PROTEINS.
AUTHOR(S): MCNUTT T L; JOHNSON L A
CORPORATE SOURCE: U.S. DEP. AGRIC., ARS, GERMPLASM GAMETE PHYSIOL. LAB., BELTSVILLE, MD., USA.
SOURCE: MEETING OF THE AMERICAN SOCIETY OF ANIMAL SCIENCE AND INTERNATIONAL SOCIETY OF APPLIED ETHOLOGY, PITTSBURGH, PENNSYLVANIA, USA, AUGUST 8-11, 1992. J ANIM SCI, (1992) 70 (SUPPL 1), 252.
CODEN: JANSAG. ISSN: 0021-8812.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L6 ANSWER 31 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 15
ACCESSION NUMBER: 92:475119 SCISEARCH
THE GENUINE ARTICLE: JH200
TITLE: BOVINE SPERMATOZOA INVITRO - A REVIEW OF STORAGE, FERTILITY ESTIMATION AND MANIPULATION
AUTHOR: COULTER G H (Reprint)
CORPORATE SOURCE: AGR CANADA, RES STN, LETHBRIDGE T1J 4B1, ALBERTA, CANADA (Reprint)
COUNTRY OF AUTHOR: CANADA
SOURCE: THERIOGENOLOGY, (AUG 1992) Vol. 38, No. 2, pp. 197-207.
ISSN: 0093-691X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 73
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In vitro storage of **bovine** spermatozoa virtually indefinitely has provided the opportunity to distribute conveniently and widely germ plasm from superior sires and benefit the productivity of **cattle** around the world. Techniques developed in our laboratories are well on their way to being able to predict accurately the fertility of young, prospective sires without the inconvenience and expense of large field trials. Manipulation of **spermatozoa** provides opportunities for the **predetermination** of **sex** of resulting offspring, the introduction of foreign DNA into oocytes, and the formation of transgenic individuals. Many other possibilities are limited only by the ingenuity of those conducting research in this exciting field.

L6 ANSWER 32 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1991-353727 [48] WPIDS
DOC. NO. CPI: C1991-152568
TITLE: Fractionating mammalian semen by treatment with antibody - selective for sperm contg. a Y chromosome, for control of sex ratios in domestic animals.

DERWENT CLASS: B04 C03 D16
INVENTOR(S): BRADLEY, M P; REED, K C
PATENT ASSIGNEE(S): (ABTE-N) AB TECHN PTY LTD
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9117188	A	19911114	(199148)*		
AU 9177579	A	19911127	(199210)		

PRIORITY APPLN. INFO: AU 1990-19 19900508
AN 1991-353727 [48] WPIDS
AB WO 9117188 A UPAB: 19930928
Semen is fractionated by contact with an antibody (Ab) which binds specifically to spermatazoa carrying a Y chromosome, then sepn. of Ab-complexed and uncomplexed sperm.

Ab is a polyclonal antibody raised in domestic fowl and able to bind to the MEA on spermatazoa. It can be labelled with a cytotoxin which inactivates sperm, or is immobilised on a solid support.

USE/ADVANTAGE - Preferential enrichment of sperm having two X-chromosomes ensures a higher proportion of female offspring (in e.g. cattle, sheep, goats, pigs, etc.). The efficiency of the fractionation process is monitored/quantified by a simple hybridisation test.

0/10

L6 ANSWER 33 OF 41 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 89:11639 CABA
DOCUMENT NUMBER: 890168664
TITLE: The technology of embryo transfer
AUTHOR: Mapletoft, R. J.
CORPORATE SOURCE: Department of Herd Medicine and Theriogenology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Sask., Canada.
SOURCE: (1988) pp. 2-39. 254 ref.
Publisher: International Embryo Transfer Society. Illinois
Meeting Info.: International Embryo Movement. Proceedings of a symposium, Montreal, 1987.
PUB. COUNTRY: United States
DOCUMENT TYPE: Conference Article
LANGUAGE: English
SUMMARY LANGUAGE: French
AB This review includes consideration of ova transfer techniques in **cattle**, **sheep**, goats and **horses**. Topics discussed include donor selection, superovulation, the recovery, handling, transfer and freezing of embryos, microsurgical procedures, genetic engineering, in vitro fertilization, **sex selection** of **spermatozoa** and embryos, and the production of chimaeras, transgenic animals and identical offspring.

L6 ANSWER 34 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1986-019961 [03] WPIDS
DOC. NO. NON-CPI: N1986-014646

TITLE: Method of regulating **sex** of **mammal** offspring - **selecting** **sperm** with **spermatozoids** travelling at speeds of 70 microns per sec. or more in sperm at 24-26 degrees celsius.
DERWENT CLASS: P14
INVENTOR(S): MALINOVSKI, A M; MAZUROVA, G M; PLATOV, E M
PATENT ASSIGNEE(S): (BREE-R) BREEDING RES
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
SU 1165334	A	19850707	(198603)*		3

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
SU 1165334	A	SU 1983-3700360	19831214

PRIORITY APPLN. INFO: SU 1983-3700360 19831214
AN 1986-019961 [03] WPIDS
AB SU 1165334 A UPAB: 19930922
The method for use especially on agricultural livestock, consists of identifying the sex-controlling spermatozoids and then selecting these for breeding. The appropriate spermatozoids are identified by the speed of their motion through the sperm fluid, and sperm is selected for insemination when it has spermatozoids with an average speed of motion of 70 microns per sec or above at a sperm temperature of 24-26 deg C, and insemination with the selected sperm is carried out on one occasion only.
ADVANTAGE - Increased output of female offspring and elimination of damage from mechanical separation of sperm.
Bul.25/7.7.85
0/0

L6 ANSWER 35 OF 41 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 86:29054 CABA
DOCUMENT NUMBER: 860194813
TITLE: Immunological sex diagnosis and sex determination in mammals: possibilities and difficulties (a review)
Immunologische Geschlechlsbestimmung und Geschlechtsbeeinflussung bei Saugetieren: Moglichkeiten und Probleme (eine Ubersicht)
AUTHOR: Walter, G.; Siems, W. E.; Nehring, H.
CORPORATE SOURCE: Zentralinstitut fur Genetik und Kulturpflanzenforschung, 4325 Gatersleben, Correnstr. 3, German Democratic Republic.
SOURCE: Biologisches Zentralblatt, (1984) Vol. 103, No. 6, pp. 613-628. 4pp. of ref.
ISSN: 0006-3304
DOCUMENT TYPE: Journal
LANGUAGE: German
SUMMARY LANGUAGE: English
AB A review of work on farm livestock and laboratory **mammals**

in which the accuracy of sex diagnosis based on detection of the H-Y antigen and of **sex** determination by **selection** of X- or Y-bearing **spermatozoa** by immunological methods is discussed. It is concluded that the former immunological technique should be used with great caution, and that immunological **selection** of **sexed spermatozoa** for animal breeding does not appear to offer much promise.

L6 ANSWER 36 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1983:142936 BIOSIS
DOCUMENT NUMBER: BR25:67936
TITLE: IMPLICATIONS FOR ARTIFICIAL INSEMINATION WITH A PRE SELECTED POPULATION OF SPERM.
AUTHOR(S): ERICSSON R J; GLASS R H
CORPORATE SOURCE: SAUSALITO, CALIFORNIA 94965.
SOURCE: NEGRO-VILAR, A. (ED.). MALE REPRODUCTION AND FERTILITY; INTERNATIONAL MEETING, MEXICO CITY. XVI+390P. RAVEN PRESS: NEW YORK, N.Y., USA. ILLUS, (1983) 0 (0), P311-318.
ISBN: 0-89004-746-4.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L6 ANSWER 37 OF 41 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 83041310 MEDLINE
DOCUMENT NUMBER: 83041310 PubMed ID: 6753153
TITLE: **Sex preselection** in **mammals**? Separation of **sperm** bearing Y and "O" chromosomes in the vole Microtus oregoni.
AUTHOR: Pinkel D; Gledhill B L; Lake S; Stephenson D; Van Dilla M A
SOURCE: SCIENCE, (1982 Nov 26) 218 (4575) 904-6.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19980206
Entered Medline: 19821218

AB The two sex determining sperm populations of the vole Microtus oregoni were separated according to DNA content by use of flow sorting instrumentation. Although the sperm were not viable, they should be useful for addressing the question of haploid expression of genes linked to sex chromosomes and for efficiently searching for biochemical markers that differentiate the two populations.

L6 ANSWER 38 OF 41 AGRICOLA
ACCESSION NUMBER: 78:153095 AGRICOLA
DOCUMENT NUMBER: 78-9142359
TITLE: **Sex selection** before fertilization [**Cattle** breeding, **spermatozoa** separation]
AUTHOR(S): Horn, P W
AVAILABILITY: DNAL (49 C29)
SOURCE: Cattleman, Sept 1978 Vol. 65, No. 4, pp. 40-42.

DOCUMENT TYPE: Journal; Article
LANGUAGE: English

L6 ANSWER 39 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1975-66933W [40] WPIDS
TITLE: Fertilised embryo transplantation technique for mammal donors - spematazoa fraction of predetermined sex character used.
DERWENT CLASS: A96 B04 B07 C03
PATENT ASSIGNEE(S): (AUGS-I) AUGSPURGER L L
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 3906929	A	19750923	(197540)*		

PRIORITY APPLN. INFO: US 1973-418604 19731123; US 1974-444022 19740220; US 1974-532253 19741212
AN 1975-66933W [40] WPIDS
AB US 3906929 A UPAB: 19930831
Embryo transplantation for increasing the number of progeny of female omnivorous and herbivorous hoofed **mammal** donors is effected by (1) inducing superovulation by administering gonadotrophin to a donor, (2) fertilising ova produced by the donor with a **spermatazoa** fraction of a desired **sex**, and (3) transplanting **selected** ova or an individual ovum derived from the donor to a selected recipient at the time when the recipient is synchronised with the development of the transplanted fertilised ovum or ova such that the transplant is synchronised at the blastocyst stage in the recipient at the time that an ovum would normally implant an ovum of its own had the recipient been fertilised.

L6 ANSWER 40 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1972-58865T [37] WPIDS
TITLE: Controlling sex of mammalian offspring - using an antibody fraction selective to only one type of sperm.
DERWENT CLASS: B04 C03
PATENT ASSIGNEE(S): (BIO-N) BIO-CONTROLS INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 3687806	A		(197237)*		

PRIORITY APPLN. INFO: US 1969-873787 19691104
AN 1972-58865T [37] WPIDS
AB US 3687806 A UPAB: 19930831
Immunological product (I) for controlling sex of **mammalian** offspring is obtd. by (a) forming a blood serum contg. sperm antibodies, each type of antibody being **selective** for 1

sperm sex type; (b) isolating a **sperm** fraction conc. inone sex type and treating this with the antibodies so taht >=80% of the antibodies reactive with the more abundant sperm type are inactivated and agglutinated, while a substantial proportion of the antibodies reactive with the less abundant sperm type are unaffected. (I), which will deactivate only 1 sperm type, may be used to treat a sperm fraction before insemination, or as a vaginal cream, is suitable for human use and stock breeding.

L6 ANSWER 41 OF 41 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1972-07929T [05] WPIDS
TITLE: Separation of **spermatozoa** cells - in a method for **pre-determining** the **sex** of an unborn **mammal**.
DERWENT CLASS: A96 B04 P14 P32
PATENT ASSIGNEE(S): (LANG-I) LANG J L; (LAN-I) LANG JL
COUNTRY COUNT: 7
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
BE 770124	A		(197205)*		
DE 2133700	A		(197305)		
NL 7109675	A		(197305)		
FR 2145760	A		(197318)		
GB 1377972	A	19741218	(197451)		
DE 2133700	B	19750123	(197505)		
CH 578873	A	19760831	(197639)		
JP 48018066	A	19730307	(198104)		
JP 55051522	B	19801223	(198104)		
NL 170803	B	19820802	(198233)		

PRIORITY APPLN. INFO: BE 1971-770124 19710716
AN 1972-07929T [05] WPIDS
AB BE 770124 A UPAB: 19930000
A fluid contg. living sperm cells which have residual positive or negative charges is treated so that cells having a certain polarity are immobilised and cells of opposite residue charge remain free and method comprises treating with an agent having an electrostatic charge of a certain polarity so that sperm cells having opposite polarity are preferentially immobilised in the fluid. Specifically claimed agents include sulphonated polystyrene polyelectrolyte, pref. (vinyl-benzene sulphonic acid sodium salt) and poly(vinylbenzyltrimethylammonium).

FILE 'HCAPLUS' ENTERED AT 14:01:01 ON 04 DEC 2002
L7 1128 S SPERM? AND (MILK OR YOLK)
L8 3 S L1 AND L7
L9 0 S L8 NOT L2

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, VETU, VETB, CABA, AGRICOLA' ENTERED AT 14:09:23 ON 04 DEC 2002)
L10 8 S L7 AND L4
L11 5 S L10 NOT L5
L12 2 DUP REM L11 (3 DUPLICATES REMOVED)

L12 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000395622 MEDLINE
DOCUMENT NUMBER: 20290354 PubMed ID: 10832757
TITLE: Insemination of mares with low numbers of either unsexed or sexed **spermatozoa**.
AUTHOR: Buchanan B R; Seidel G E Jr; McCue P M; Schenk J L; Herickhoff L A; Squires E L
CORPORATE SOURCE: Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins 80523, USA.
SOURCE: THERIOGENOLOGY, (2000 Apr 1) 53 (6) 1333-44.
Journal code: 0421510. ISSN: 0093-691X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000817

AB Two experiments were conducted to determine pregnancy rates in mares inseminated 1) with 5, 25 and 500 x 10(6) progressively motile **spermatozoa** (pms), or 2) with 25 x 10(6) sex-sorted cells. In Experiment 1, mares were assigned to 1 of 3 treatments: Group 1 (n=20) was inseminated into the uterine body with 500 x 10(6) pms. Group 2 (n=21) and Group 3 (n=20) were inseminated into the tip of the uterine horn ipsilateral to the preovulatory follicle with 25 and 5 x 10(6) pms, respectively. Mares in all 3 groups were inseminated either 40 (n=32) or 34 h (n=29) after GnRH administration. More mares became pregnant when inseminated with 500 x 10(6) (18/20 = 90%) than with 25 x 10(6) pms (12/21 = 57%; P<0.05), but pregnancy rates were similar for mares inseminated with 25 x 10(6) vs 5 x 10(6) pms (7/20 = 35%) (P>0.1). In Experiment 2, mares were assigned to 1 of 2 treatments: Group A (n=11) was inseminated with 25 x 10(6) **spermatozoa** sorted into X and Y chromosome-bearing populations in a skimmilk extender. Group B (n=10) mares were inseminated similarly except that **spermatozoa** were sorted into the skimmilk extender + 4% egg **yolk**. Inseminations were performed 34 h after GnRH administration. Freshly collected semen was incubated in 224 microM Hoechst 33342 at 400 x 10(6) **sperm**/mL in HBGM-3 for 1 hr at 35 degrees C and then diluted to 100 x 10(6) **sperm**/mL for **sorting**. **Sperm** were **sorted** by **sex** using flow cytometer/cell **sorters**. **Spermatozoa** were collected at approximately 900 cells/sec into either the extender alone (Group A) or extender + 4% egg **yolk** (Group B), centrifuged and suspended to 25 x 10 **sperm**/mL and immediately inseminated. Pregnancy rates were similar (P>0.1) between the **sperm** treatments (extender alone = 13/10, 30% vs 4% EY + extender = 5/10, 50%). Based on ultrasonography, fetal sex at 60 to 70 d correlated perfectly with the sex of the **sperm** inseminated, demonstrating that foals of **predetermined sex** can be obtained following nonsurgical insemination with sexed **spermatozoa**.

L12 ANSWER 2 OF 2 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 85:100701 CABA
DOCUMENT NUMBER: 850190853

TITLE: **Preselection** of **sex** of lambs by layering **spermatozoa** on protein columns

AUTHOR: White, I. G.; Mendoza, G.; Maxwell, W. M. C.
CORPORATE SOURCE: Department of Veterinary Physiology, University of Sydney, Sydney, New South Wales 2006, Australia.

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AB Semen from 3 Merino rams was diluted to 200 x 106 **spermatozoa**/ml in fructose-citrate; 2 ml of diluted semen was layered onto a column of 6 ml of Tris-glucose-citrate-bovine serum albumin. **Spermatozoa** were recovered by centrifugation from the top and bottom of the column after 2 h, reconstituted in fructose-citrate, recentrifuged and diluted with Tris-glucose-citrate-egg **yolk**-glycerol. They were then frozen in pellets on dry ice until used for insemination of 87 Merino ewes that had been synchronized with Chronogest intravaginal sponges. The percentages of male and female lambs born were 36.4 and 63.6 resp. for ewes inseminated with **spermatozoa** from the top of the column vs. 75.0 and 25.0 for ewes inseminated with semen from the bottom of the column..

(FILE 'MEDLINE' ENTERED AT 14:30:59 ON 04 DEC 2002)

L13	519	SEA	FILE=MEDLINE	ABB=ON	PLU=ON	"SEX PRESELECTION"/CT
L14	30229	SEA	FILE=MEDLINE	ABB=ON	PLU=ON	SPERMATOZOA/CT
L15	111	SEA	FILE=MEDLINE	ABB=ON	PLU=ON	L13 AND L14
L16	13105	SEA	FILE=MEDLINE	ABB=ON	PLU=ON	MAMMALS/CT
L17	4	SEA	FILE=MEDLINE	ABB=ON	PLU=ON	L16 AND L15

L17 ANSWER 1 OF 4 MEDLINE
AN 2000199254 MEDLINE
TI Sex preselection: high-speed flow cytometric sorting of X and Y sperm for maximum efficiency.
AU Johnson L A; Welch G R
SO THERIOGENOLOGY, (1999 Dec) 52 (8) 1323-41. Ref: 49
Journal code: 0421510. ISSN: 0093-691X.

AB Sex preselection that is based on flow-cytometric measurement of sperm DNA content to enable sorting of X- from Y-chromosome-bearing sperm has proven reproducible at various locations and with many species at greater than 90% purity. Offspring of the predetermined sex in both domestic animals and human beings have been born using this technology since its introduction in 1989. The method involves treating sperm with the fluorescent dye, Hoechst 33342, which binds to the DNA and then sorting them into X- and Y-bearing-sperm populations with a flow cytometer/cell sorter modified specifically for sperm. Sexed sperm are then used with differing semen delivery routes such as intra-uterine, intra-tubal, artificial insemination (deep-uterine and cervical), in vitro fertilization and embryo transfer, and intra-cytoplasmic sperm injection (ICSI). Offspring produced at all locations using the technology have been morphologically normal and reproductively capable in succeeding

generations. With the advent of high-speed cell sorting technology and improved efficiency of sorting by a new sperm orienting nozzle, the efficiency of sexed sperm production is significantly enhanced. This paper describes development of the these technological improvements in the Beltsville Sexing Technology that has brought sexed sperm to a new level of application. Under typical conditions the high-speed sperm sorter with the orienting nozzle (HiSON) results in purities of 90% of X- and Y-bearing sperm at 6 million sperm per h for each population. Taken to its highest performance level, the HiSON has produced X-bearing-sperm populations at 85 to 90% purity in the production of up to 11 million X-bearing-sperm per h of sorting. In addition if one accepts a lower purity (75 to 80%) of X, nearly 20 million sperm can be sorted per h. The latter represents a 30 to 60-fold improvement over the 1989 sorting technology using rabbit sperm. It is anticipated that with instrument refinements the production capacity can be improved even further. The application of the current technology has led to much wider potential for practical usage through conventional and deep-uterine artificial insemination of many species, especially cattle. It also opens the possibility of utilizing sexed sperm for artificial insemination in swine once low-sperm-dose methods are perfected. Sexed sperm on demand has become a reality through the development of the HiSON system.

L17 ANSWER 2 OF 4 MEDLINE
AN 2000157266 MEDLINE
TI Sexing mammalian spermatozoa and embryos--state of the art.
AU Seidel G E Jr
SO JOURNAL OF REPRODUCTION AND FERTILITY. SUPPLEMENT, (1999) 54 477-87.
Ref: 68
Journal code: 0225652. ISSN: 0449-3087.
AB Methods for sexing preimplantation embryos range from karyotyping to recording speed of development in vitro. The only method used routinely on a commercial scale is to biopsy embryos and amplify Y-chromosome-specific DNA using the polymerase chain reaction. This method is effective for more than 90% of embryos and is > 95% accurate. Within males, spermatozoa are essentially identical phenotypically due to: (1) connection of spermatogenic cells by intercellular bridges, (2) transcriptional inactivation of sex chromosomes during meiosis and spermiogenesis, (3) severe limitation of all gene expression during the later stages of spermiogenesis, and (4) coating all spermatozoa with common macromolecules during and after spermiogenesis. One consequence is that no convincing phenotypic difference has been detected between X- and Y-chromosome-bearing spermatozoa. The only consistently successful, nondestructive approach to sexing spermatozoa is to quantify DNA in spermatozoa using a fluorescing DNA-binding dye followed by flow cytometry and cell sorting. X-chromosome-bearing ruminant spermatozoa have about 4% more DNA compared with Y-chromosome-bearing spermatozoa; accuracy of sorting can exceed 90% routinely, and sorting rates currently exceed 10(3) live spermatozoa of each sex chromosome composition s-1. Hundreds of apparently normal offspring from a number of species have been produced from sexed semen, some via intrauterine artificial insemination.

L17 ANSWER 3 OF 4 MEDLINE
AN 91375487 MEDLINE
TI [Molecular mechanisms of regulating mammalian sex and problems of

fractionating spermatozoa].
Molekuliarnye mekhanizmy reguliatsii pola u mlekopitaiushchikh i problema fraktsionirovaniia spermatoidov.
AU Popov L S; Rekesh A N
SO MOLEKULIARNAIA BIOLOGIIA, (1991 Jan-Feb) 25 (1) 22-42. Ref: 125
Journal code: 0105454. ISSN: 0026-8984.
AB We present results of a study devoted to genetic determination and to the mechanism of primary sex differentiation in mammals. Progress is achieved in the mapping of a Y chromosome region necessary and sufficient for testis determination in man (TDF) and mouse (Tdy). We discuss a possible role in sex regulation of a recently described highly conservative locus from this region, ZFY (and similar loci within other chromosomes probably coding for Zn-binding proteins, transcription regulators) and H-Y antigen as well. We note that neither locus ZFY nor H-Y can play the role of TDF (Tdy) and that studies in this direction should be carried out. Numerous works on fractioning according to sex of spermatozoa of mammals including man are critically reviewed. Contradictory data exist in literature concerning the applicability of different approaches for this purpose: from fractioning based on different inherent mobility of different sex cells or gel-filtration, to the sorting in a flow cytometer equipped with a laser light source and a computer. However, in many cases the principle underlying this or that method of fractionation and determining the positive results, i.e. the statistically important shift of the sex ratio as compared with the initial sperm, remains unclear. It is stated that on the immunological and electrophoretic approaches might appear most promising for practical application, and in cattle-breeding as well. Modern procedures for sex testing and the fertility control of spermatozoa are also examined.

L17 ANSWER 4 OF 4 MEDLINE
AN 80074798 MEDLINE
TI Selecting a mammalian species for the separation of X- and Y-chromosome-bearing spermatozoa.
AU Moruzzi J F
SO JOURNAL OF REPRODUCTION AND FERTILITY, (1979 Nov) 57 (2) 319-23.
Journal code: 0376367. ISSN: 0022-4251.
AB All (524) male karyotypes in An Atlas of Mammalian Chromosomes (Hsu & Benirschke, 1967-1977) were visually estimated for the chromatin difference between the X- and Y-chromosome-bearing spermatozoa. After more exact measurement of axial chromatid length for 100 karotypes, 24 species were found in which the difference between X and Y chromosomes was greater than 6.2%. It is suggested that such species would be the best for attempts to separate the X- and Y-bearing spermatozoa.

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